REQ. NO. 3-9188

CONTRACT REPORT NO. 1SQ83-00188

"BIOLOGICAL CONTROL OF METHANE IN COAL MINES - A FEASIBILITY STUDY"

A REPORT
BY

GEMINI BIOCHEMICAL RESEARCH LTD.

Work on this project was conducted under
the auspices of the Canada centre for Mineral
and Energy Technology, Energy Mines and Resources, Canada

D.S.S. File Number: 03SQ.23440-3-9188

D.S.S. Contract Serial Number: 1SQ83-00188

Canmet Project Number:

Canmet Scientific Authority: Dr. R.N. Chakravorty

79 pp

FEB 07 1984
MICROMEDIA

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INTRODUCTION AND WORK PLAN

In response to discussions with Dr. R. N. Chakravorty of CANMET, Department of Energy, Mines and Resources on the potential use of methane oxidizing microorganisms the following work plan was established to guide this study into the feasibility of biological control of methane in coal mines.

BACKGROUND

Most coal mine explosions have been caused by the emission and accumulation of methane. In spite of major advances in the mining technology it has not been possible to eliminate gas explosions. In Canadian coal mines the problem of methane emission could be quite serious as the productivity increases and the workings become more extensive. Research for the effective control of methane in all phases of mining is therefore considered essential.

A review of techniques developed for dealing with methane in coal mines indicates that control by ventiliation alone may not be the answer to all mining situations. Alternate methods such as methane drainage during mining operations or in advance of mining showed promising results. However, for control of methane in the worked out areas or in coal silos it is felt that unconventional techniques based on biological control may prove useful and practical.

Though considerable research efforts are being directed towards methane control by ventilation and drainage, so far little attention has been paid on the application of biological techniques for removing methane. This contract is specially directed towards evaluating methane control using methane oxidizing bacteria, keeping in view their possible application in Canadian mining operations. A critical appraisal of this technique is considered important to achieve CANMET objective towards safer working environment in coal mines.

WORK STATEMENT:

a) Prepare a state of the art review on the biological control of methane in coal mines.

b) Identify the parameters which may help or retard the activity

of methane oxidizing bacteria under actual mining conditions.

c) Identify limitations to the application of this technology in underground coal mining situations i.e. rapid advance of face, removal of methane from mined out areas, etc.

d) Evaluate possible application of this technique for controlling methane during storage and transportation of coal (e.g. coal silo, ship hold).

e) Present an in-house seminar at Ottawa highlighting the merits and demerits of this technique for application in coal mines. Recommend measures for future R & D work.

This report was prepared by Drs. B.G. Thompson and P.I. Forrester of Gemini Biochemical Research Ltd.

1.0 BIOLOGICAL METHANE CONTROL

Methane levels in coal mines, coal storage and coal transportation systems can be controlled when necessary by ventillation, pre-drainage of the methane from the coal seam prior to or during mining operations, or by water infusion of the coal face in the coal mine. (Zabetakis 1973, Prosser et al. 1981, Cervik and Cetinbus 1973, Suarez and Chakravorty 1976, Chakravorty 1983). An alternative biologically enhanced method for the control of methane emitted from coal has been suggested and studied, particularly in Eastern Europe, that may provide a way of at least enhancing the methane control achieved by conventional methods (Masszi, 1982). In general the method would involve bringing methane oxidizing microorganisms (MOM'S) in contact with the coal and allowing them to oxidize the methane to non-flammable product, carbon dioxide, in a controlled manner.

However, little field testing of this biological process has been carried out. To date, two groups of workers have reported on actual mine testing of biological methane control. One of these groups is in Russia and the other in India.

Preliminary studies performed in coal mines have indicated that biologically enhanced methane control has possible uses in coal mines. Ivanov et al. (1981) treated a 30m x 20m surface section of coal adjacent to a coal longwall with a fermenter grown culture of a methane oxidizing bacterium Methylococcus capsulatus. In this mine 70% of the methane came from worked out areas. Over a 15 day period the bacterium oxidized from 6.3% of the methane emitted from the treated area on day 1, to a high of 40% by day 9. By day 15 the amount of methane oxidized had fallen to 13% of the total methane emitted by the treated area. The actual volumes of outgassed methane were not reported (Ivanov et al. 1981). Abramov et al. (1979) found that the bacterium Methylomonas methanica could over an unspecified time, oxidize all the methane in a coal seam after the organism had been injected into the seam.

Thakur and co-workers (Thakur et al. 1982) in India also carried out field testing in three different mines. In one mine there was an approximate 50% decrease in emission of methane three days after a nutrient solution and MOM'S were injected into the coal seam. In a second test there was complete removal of methane two months after injection of bacteria and nutrients. In the third test the methane emission rate was reduced by approximately 66% 20 days

after injection of the organisms. These authors did not add oxygen to the coal and claim to have isolated an anaerobic strain of organism. However, they did not give any details on the apparent mechanism of this reaction. A survey of their medium constituents would suggest that sulphate is being used as the terminal electron acceptor but there is no indication on the fate of the sulphate ions (see section 2.2.9).

This report shall evaluate the factors affecting the potential use of methane oxidizing microorganisms for biological methane control.

2.0 PARAMETERS WHICH MAY HELP OR RETARD THE USE OF MOM'S IN ACTUAL MINING CONDITIONS

2.1 Isolation of MOM'S and Other Organisms from Coal Mines

The existance of methane oxidizing bacteria was first noted by Kaserer in 1905 (Kaserer, 1905). Sohngen in 1906 (Sohngen, 1906) was the first to isolate an organism able to grow on methane as a carbon source. Since that time a large number of methane oxidizing bacteria, many of them uncharacterized, have been isolated (Wolfe and Higgins, 1979). A limited number of non-bacterial microorganisms capable of oxidizing methane have also been described (Table 3).

Methane oxidizing bacteria have been isolated from a large variety of sources including soils, marshes, muds, sewage, plants, fresh and salt water sources, and coal mines (Table 4). The very wide range of habitats and locations that these organisms have been isolated from suggests that these organisms could be considered upiquitous.

The number of yeast, fungi, and algae that have been described that oxidize methane are limited. They have been isolated from lakewater, sewage, mud, and soil (Table 5).

The success rate for the isolation of methane oxidizing microorganisms where reported is high. Smirnova and Golubeva (1969) reported that of 109 samples taken from a variety of non-mine sources (Table 6), 78 or 71.6% of the samples contained methane oxidizing bacteria. Hutton and Zobell (1949) reported that of 299 samples taken from a variety of non coal mine sources, 209 or 69.9% of the samples contained methane oxidizing bacteria (Table 7). In both cases samples were primarily taken from areas where hydrocarbon sources or sources of ground methane would be expected to be present.

Reports of the isolation of methane oxidizing bacteria from coal mine environments are limited and originate from Eastern Europe. Malashenko and Romonovskaya (1978) examined 500 samples from coal mines and soils in the U.S.S.R. and found that most (unspecified) of the samples contained methane oxidizing bacteria. Ivanov et al. (1978) reported that of 76 water samples taken from 14 coal mines in the U.S.S.R. 57 or 75% of the samples contained methane oxidizing bacteria. A polish study (Goldlewska - Lipowa, 1979) found that all of 200 water samples taken from Polish coal mines were found to contain methane oxidizing bacteria. The isolation regime that led to this high success rate included simulating the temperature, pH, and ionic constitution of the mine waters involved in the isolation

media used in the laboratory. These studies suggest that conditions suitable for supporting the growth of methane oxidizing bacteria exist in many coal mines.

Canadian studies on the isolation of methane oxidizing bacteria are limited. Mueller (1969 a, b) has found methane oxidizing bacteria in sewage and Roth-Wood (1983) has found them in soils in southern Alberta.

Thus MOM'S are found to occur in a widespread variety of habitats including coal mines.

Conditions in coal mines have been shown to be favourable for the growth of a wide variety of non-methane oxidizing bacteria and fungi. Rogoff et al. (1962) in a review described a large number of bacteria and fungi found associated with all grades of coal. A summary table from this review is included (Table 8). These organisms were all found to have normal habitats in nature outside of the coal mine and were concluded to have entered the mine from outside sources such as mine waters or the rock overburden above the seam (Rogoff et al. 1962). The isolation of such organisms indicates that other organisms such as methane oxidizing microorganisms that are common in non-mine habitats may gain entry to coal mines in a similar fashion.

2.2 Metabolic and Physical Characteristics of MOM'S and their Relationship to Potential Uses for Biological Methane Control

2.2.1 Overview

The metabolic and physical characteristics of MOM'S will effect any potential use for the biological control of methane. In general the organisms must be able to grow and oxidize methane in the environment of coal without adversely effecting the coal.

Specifically any potentially useful MOM must:

1. be able to grow and oxidize methane at the ambient temperatures, pH's, pressures and ionic conditions found in coal.

2. be able to grow and oxidize methane in the presence of any organic constituents of the coal.

3. have access to a suitable electron acceptor for the oxidation of methane.

4. be able to be applied to coal or into coal without being adversely affected.

5. not adversely affect the structure of the coal.

This section will review the metabolic and physical properties of methane oxidizing microoganisms as they relate to the described features.

2.2.2 Obligate vs. Non-Obligate Methanotrophy and Potential Effects on Coal Structure by MOM'S

The metabolic properties of MOM'S are very diverse. In general they can be characterized as obligate or non-obligate methanotrophs. Obligate methanotrophs are those microorganisms that can only utilize small, generally 2 carbon or less, compounds as sole carbon sources. Most obligate methanotrophic bacteria share the metabolic properties outlined in Table 1 (Wittenbury et al. 1976). Non-obligate methanotrophs have the ability to utilize larger (more than 2 C) carbon sources as their sole carbon source as well as being able to oxidize methane as a sole carbon source. Examples of non-obligate methanotrophic bacteria and some of the 3 carbon or greater compounds they can oxidize are found in Table 9. The metabolic properties of these bacteria vary greatly as a group.

The non-bacterial MOM'S examined can be categorized as non-obligate methanotrophs. All 5 strains of yeast isolated by Wolf and Hanson (1979) grew on compounds such as acetate, ethane, glucose, and butane as well as methane. The methane oxidizing fungi examined by Zajic et al. (1969) grew on glucose, sucrose, and maltose. The 3 strains of yeast isolated by Smirnova and Golubeva (1969) required biotin as well as methane for growth.

The ability of some non-obligate methanotrophs to oxidize large compounds of size greater than 2 carbon may prove detrimental to the coal structure. Coal is composed of a complex arrangement of polyaromatic and polycycloparaffin groups often linked by aliphatic groups, all substituted extensively with sulphur, oxygen and nitrogen containing groups (Larsen, 1981). The ability of MOM'S to utilize coal components has not been examined. However studies have shown that bacteria, yeast, and fungi can oxidize a wide variety of saturated and unsaturated aliphatic compounds, aromatic compounds, and cycloparaffins (Rogoff et al. 1962, Davis 1956). Methane oxidizing bacteria have been shown to oxidize long chain aliphatic mixtures, petroleum fractions like kerosene that contain aromatics, phenol, and napthalene (Table 8). Any microorganisms used for biological methane control that must come in contact with the coal must therefore be considered to be potentially able to degrade coal. The use of obligate methanotrophs exclusively would avoid any potential coal degradation.

Table 1: Properties of obligate methanotrophic bacteria

1. Gram-negative strictly aerobic; rods, vibrios, or cocci.

2. Catalase-positive, oxidase positive, and possess cytochromes of c, a, or b type.

3. To some degree sensitive to normal oxygen tension in air. Some will not grow in shaking flasks. If N_2 fixation is induced, they become extremely oxygen sensitive.

Carbon Sources

4. All use methane, methanol, dimethyl ether, methyl formate, dimethyl-carbonate, and (those tested) formaldehyde as sole carbon and energy sources.

5. Carbon dioxide, acetate, and some amino acids are used as supplementing carbon sources. Carbon compounds cooxidized are also likely to provide supplementary carbon.

Nitrogen Sources

6. All use ammonia; most use nitrate and nitrite. Some use urea, amino acids, and yeast extract. All those with serine pathway fix dinitrogen.

7. All reduce nitrate to nitrite, but will not grow anaerobically on CH_4 because O_2 is required.

Cooxidation

8. All oxidize ammonia, carbon monoxide, dimethyl ether, propane, ethane, ethanol propanol, butanol, formaldehyde, and formate, among other compounds.

Morphology

9. All possess a complex internal arrangement of paired membranes.

10. All form a differentiated resting body (exposure or cyst).

1: from Whittenbury et al. (1976)

2.2.3 Methane Oxidation Rates

The rate at which methane is oxidized by microorganisms will vary with culture conditions, the state of growth of the microorganisms and the type of microorganisms. Cooney and Levine (1972) describe a continuous culture system where the bacteria, under optimal growth conditions, oxidized 5.83 g methane /h/ litre of culture and had a productivity of 3.5 g of cells /h/ litre of culture. Sheehan and Johnson (1971) also describe a continuous culture system where the bacteria oxidized 3.88 g methane /h/ litre of culture, had a productivity of 2.39 g cells /h/ litre of culture and a doubling time of 2.29 h. Clearly, under optimal conditons some methane oxidizing bacteria are capable of high methane oxidation rates. The activity of these organisms under sub-optimal conditons was not investigated.

The methane utilizing yeasts described oxidized methane at rates slower than those reported for bacteria. Wolf and Hanson (1979) reported that 5 yeasts were capable of oxidizing up to 0.6 g methane /h/ litre of culture.

The rate at which microorganisms will demethanate a coal will therefore depend on the rate of methane oxidation, the amount of culture applied to the coal, and the amount of methane found in the coal. Coals mined in the United States have methane contents ranging from nearly zero to 12.30 g /kg coal (Diamond 1978). Coal mines in Canada can contain coal with similar levels of methane. The mine at Cardinal River, Alberta has 12.24 g methane /kg coal (Rogan 1983). The water content of coals after water infusion operations used to limit dust problems is about 3% (w/w) (Deprez 1978). If 3% (w/w) of a bacterial culture oxidizing 5.83 g methane /h/ litre or a yeast culture oxidizing 0.6 g methane /h/ litre are infused into this coal it would take 2.93 and 28.47 days, respectively, for these cultures to remove all the methane.

2.2.4 Temperature and pH Effects

Methane oxidizing bacteria have been shown to grow over a wide range of temperatures and pH's. Temperatures reported to support growth by methane oxidizing bacteria range from 15° C to 62° C (Table 10). Most of these bacteria will not grow over this entire range of temperature and are restricted to growth between narrow temperature limits (Table 10) spanning up to 25° C. Methane oxidizing bacteria have been reported to grow over a pH range of 3.0 to 10.0 (Table 11).

The non-bacterial methane oxidizing microorganisms that have been identified, grow at temperatures and pH range similar to those of bacteria (Table 12). The number of organisms described is small and varied.

The specific range of temperature and pH tolerance of each organism selected for biological methane control applications would be matched with that of the environmental conditions found in the mines selected for microbiological treatment.

The pH of water in coals will affect the activity of methane oxidizing microorganisms placed in contact with coal. In coals with high sulphide content, the pH of the water in the coal may dip below pH 2 (Harrison 1978). This is due to the presence of sulphide oxidizing bacteria that convert sulphides to sulphuric acid (Vishniac and Santer 1957). These pH values are below those reported for the range of growth for methane oxidizing microorganisms (Tables 11 and 12). However, Ivanov et al. (1978) found that the pH of waters found in coal from 7 mine sources ranged from 6.6 to 8.8, which is within the range of pH values found for the growth of MOM's (Tables 11 and 12). All of these samples contained methane oxidizing bacteria.

The temperatures of coal mines, coal storage and coal transportation systems may also affect the growth of methane oxidizing microorganisms. Particularly in winter conditions, coal storage and shipping systems may have temperatures below 15° C. Most organisms studied to date have been mesophilic methane oxidizers i.e. their temperature optima range from 15 to 35° C. However, another group of organisms, the psychrophilic group, are active at temperatures between 0 and 15° C. These organisms can be isolated from the sediments of cold lakes or arctic deltas.

2.2.5 Pressure Effects

The ability of MOM'S to tolerate and grow at high pressure has not been examined in depth. Hutton and Zobell (1949) isolated a methane oxidizing bacterium from marine sediments that had been under 117 atmospheres (1720 psi) of hydrostatic pressure. The organism was isolated at normal air pressure and no effort was made to grow the organism at in situ pressure. A methane oxidizing bacterium, Methylomonas methanica, that had been isolated at normal air pressure, has been shown to retain the ability to grow after being injected into a coal seam at pressures up to 150 atmospheres (Abramov et al. 1977).

The study of the resistance of microorganisms to pressure in general has led to several conclusions. Zobell and Johnson (1949) demonstrated that of a large number of randomly chosen terrestrial bacteria and yeast whose normal growth pressure was 1 atmosphere or less, most could grow at 300 atmospheres and 30° C. as well they could grow at 1 atmosphere (Table 13). As pressures increased beyond this level the ability to grow declined in most cases. However, at higher pressures, while many bacteria do not grow, they retain their

viability and will grow again if the pressure is dropped. The yeast examined did not demonstrate this trait.

The resistance of MOM'S to pressure is important to potential uses for biological methane control in several respects. In coal faces the gas pressures can increase rapidly from atmospheric at the face to high levels only a short distance into the seam.

For example at a depth of 37 m from the face in the Pocahontas #3 mine, U.S.A., the gas pressure was found to be 650 psi or 44.2 atm. (Kissell 1973). Microorganisms injected into these conditions would have to be pressure tolerant.

In addition, organisms applied to coal under certain conditions would have to be pressure tolerant. If organisms were applied to coal during hydraulic mining they would need to be able to tolerate, but not grow at, pressures up to 1000 psi (Chakravorty 1983) and be able to survive rapid decompression back to atmospheric pressure after application. ZoBell and Johnson (1949) determined that many bacteria can survive pressure and decompression cycles at pressures up to 600 atmospheres (Table 13). Organisms injected in fluids either to increase infusion rates (see section 3.1) or to increase the amount of oxygen dissolved in the fluid (see section 2.2.8) would also need to be pressure resistant.

2.2.6 Ion Effects and Requirements

A summary of the variety of ions which have been used in the culturing of MOM'S is shown in Table 14. In most cases which ions are essential for the growth of the organisms have not been determined. Many of the bacteria described have been grown on media lacking many of these ions. It is probable that the ionic requirements of methane oxidizing bacteria vary from organism to organism and would have to be determined for each separate case.

Certain ions have been shown to be inhibitory to the growth of methane oxidizing bacteria at certain concentrations. NH_4Cl at concentrations greater than 10 mM can inhibit the growth of Pseudomonas methanica (Ferenci et al. 1974). This ion is a common source of nitrogen for many methane oxidizing bacteria. Phosphate ion concentrations of >0.28 M inhibited the growth of Methylococcus capsulatus (Harwood and Pirt 1972). Concentration of this ion <0.28 M stimulated the growth of the organism (Harwood and Pirt 1972). Cu^{2+}, another common ion found in many media used for propagating methane oxidizing bacteria was found to inhibit the growth of a methane oxidizing Bacillus sp. at concentrations (as $CuSO_4 \cdot 5H_2O$) greater than 20 mg/L (Wolnak et al. 1967). The ion species that can adversely effect the activity of methane oxidizing bacteria probably vary from organism to organism

and many ion species that are necessary for growth at low concentrations, may inhibit growth at higher concentrations. Minimum ionic requirements would have to be determined in each separate case.

The ionic requirements for the non-bacterial MOM'S are similar to those described for bacteria (Table 14).

Coal can contain a wide range of non-organic compounds. For example Kessler et al. (1973) analysed 13 coals ranging in rank from anthracite to very high volatile content coals and found 64 elements in the coal ranging in concentration from 0.01 to 41,000 ppm.

The inorganic fraction of coal is composed largely of minerals with lesser amounts of organo-metallic compounds and cations bound ionically to the coal (Cheng Tsai 1982). A representative list of the minerals found in coals is found in Table 14 (Francis 1961).

Many of the ionic constituents in coal have been shown to be accessible to aqueous solutions applied to coal. Bettelheim and Hann (1980) have found up to 40% of the chloride ion found in British coals is extractable into water. Khenkina et al. (1977) found large variations in the levels of ions extractable from coal with water. The most abundant ions in the extracts were SO_4, Cl^-, and HCO_3; Mg^{2+}, PO_4^{3-} and nitrogen containing ions were present in negligible amounts (Khenkina et al. 1977). The concentrations of these ions in the extracts affected the growth and activity of methane oxidizing bacteria grown in the extracts and as a result the authors concluded that the effects of each ion found in aqueous extracts be studied prior to any tests of in situ methane oxidation. Kurdish et al. (1978) also found that the concentration of ion species extractable from coal with water varies from coal to coal (Table 2). The extracts were lacking in phosphate and nitrogen containing ions (Table 2). Kurdish et al. (1978) demonstrated that the aqueous extracts from these coals affected the methane oxidizing activity of Methylomonas methanica, Methylomonas ucrainicus, and Methylococcus thermophilus in a random fashion both positively and negatively.

The variation of ions and elemental species available in coal to microorganisms, and their toxic effects MOM'S make predictions of the effects of a specific coal on the activity of a specific microorganism impossible. Each case will have to be examined individually. However, bacterial, fungal and yeast species isolated from coal will be already adapted to their environmental conditions and would be the easiest to evaluate.

2.2.7 Effect of Organic Components of Coal

The activity of methane oxidizing microorganisms may be affected by being in physical contact with the coal or be affected

Table 2 - Ionic species extracted by water from various coals.[a,b]

Ion	Coal 1	2	3	4
SO_4^{2-}	25.3	246.8	13.5	2.2
Cl^-	58.5	53.1	28.6	43.6
HCO_3^-	108.6	74.0	89.6	89.6
NO_2^-	0	0	0	0
NO_3^-	0	0.2	0	0
PO_4^-	0	0	0	0
Na^+	5.1	42.5	22.5	37.3
K^{2+}	0.6	0.6	0.4	0.3
Fe^{+3}	0.011	0.015	0.010	0.011
Ca^{2+}	17.6	29.5	21.6	9.8
NH_4^+	0	3.7	0	0
Cu^{2+}	30.0	15.0	15.0	40.0
Fn^{2+}	140.0	280.0	150.0	180.0

a from Khurdish et al (1976)
b Concentrations expressed as mg/L extract.

by the organic components of the coal.

The physical adsorbtion of methane oxidizing bacteria to coal has been shown to stimulate methane oxidation by bacteria. Methylosinus trichosporium and Methylococcus ucrainicus when adsorbed to anthracite coal oxidized 2.1 x and 1.5 x, respectively, the methane of an equal amount of non-adsorbed bacteria (Nesterov and Nazerenko 1975). Methylomonas methanica has also been shown to oxidize 1.14 x more methane when adsorbed to coal than in the free state (Abramov et al. 1977). The coal was suggested to have acted as an inert chemical adsorbant that did not effect the bacteria adversely and stimulated methane oxidation (Nesterov and Nazerenko 1975).

The stimulative effect of coal on the activity of methane oxidizing bacteria and the findings that fungi, yeast, and bacteria can all be isolated from coal surfaces suggest that the organic compounds found in coal have little effect on the growth of microorganisms. This may be related to the limited solubility of these compounds in water. Methanol extracts of coal have been shown to be inhibitory to microbiological growth (Rogoff et al. 1962).

2.2.8 Oxygen Requirements

The complete oxidation of methane to carbon dioxide by MOM'S that utilize oxygen as a terminal election receptor is governed by the following equation:

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O$$

This reaction only holds true if the methane is completely oxidized to carbon dioxide. The methane oxidizing microorganisms assimilate some of the carbon from the methane into their biomass and this assimilated carbon is not as highly oxidized as carbon dioxide. Hence MOM'S will consume less than 2 moles of oxygen/mole methane oxidized. The reported range is from 0.29 to 1.8 moles oxygen reduced/mole of methane oxidized by the microorganisms (Table 16).

The amount of oxygen utilized may depend on the strain of microorganism used, the physiological state of the cells, and whether or not the cells are grown under oxygen or methane limited growth conditions. Studies on the methane oxidizing bacterium M102 demonstrated that actively growing cultures that are increasing in total biomass, consumed 1.1 to 1.2 moles of oxygen/mole of methane oxidized (Naguib 1976). Resting cultures or cultures not incorporating methane into biomass consumed 1.4 to 1.8 moles of oxygen/mole of methane oxidized. Brown et al. (1964) found the same pattern of oxygen consumption in 4 unidentified cultures of methane oxidizing bacteria. Some bacteria utilize the same amount of

oxygen/mole of methane consumed under either oxygen or methane limited growth conditions (Vary and Johnson 1967, Nagai et al. 1973) . However, Harwood and Pirt (1972) reported that Methylococcus capsulatus utilized 0.29 to 0.31 moles of oxygen/mole of methane under oxygen limitation, and utilized 1.8 mole of oxygen/mole of methane consumed under methane limitation.

The use of microorganisms that utilize oxygen as a terminal electron acceptor necessitates that enough oxygen be available to the organisms to oxidize all the methane. A range of 0.29 to 1.8 mole oxygen per mole of methane oxidized will be used for the next part of the discussion.

Four different situations were evaluated for the availability of oxygen for biological demethanation. These cases were:

(i) interior of a coal seam

(ii) surface of a coal seam

(iii) sealed storage or transportation systems

(iv) ventilated storage or transportation systems

The results of the evaluation are summarized as follows. In general, the interior of a virgin coal seam would contain little or no oxygen thus there would be little methane oxidation unless oxygen was injected into the coal. Best results would be obtained by applying the MOM'S to the surface of the coal. An air velocity of 0.7 m/h would be sufficient to supply enough oxygen to allow the organisms to reach their demethanating capacity. This is much lower than the average rates of between 146 and 730 m/h commonly found in mines (Chakravorty 1983).

In the case of sealed or air tight storage or transportation systems there would not be sufficient oxygen present to dementhanate the coal unless there was less than 2% (v/v) of coal in the system. In systems in which there was limited access of air then the levels of methane could be controlled.

2.2.9 Anaerobic Methane Oxidation

The majority of methane oxidizing bacteria examined and all of the methane oxidizing yeasts and fungi have been shown to use oxygen as the terminal electron acceptor during the oxidation of methane. The products of this reaction are carbon dioxide, cell biomass, and water. If these microorganisms were to be used for biological methane control up to 1.8 moles oxygen/mole methane oxidized must be supplied to the microorganisms (Section 2.2.8).

The anaerobic oxidation of methane would allow demethanation in the absence of oxygen. This would eliminate any potential problems associated with the use of oxygen in a high methane coal environment.

Both the nitrate and sulphate ion have been reported as alternative terminal electron acceptors during the anaerobic oxidation of methane.

Zajic and Smith (1966) demonstrated that, in Pseudomonas methanica (Temple), nitrate could serve as the terminal electron acceptor during the methane oxidation. The reaction is favourable thermodynamically (Zajic and Kosaric 1974).

$$3CH_4 + 2NO_3^- \longrightarrow 3CO_2 + N_2 + 6H_2 \qquad \Delta G = -129 \text{ Kcal/mole}$$

(NO_3^- as KNO_3)

Davis and Yarbrough (1966) found that a strain of Desulphovibrio desulphuricans isolated from a methane bearing aquifer in Texas could utilize sulphate ion as a terminal electron acceptor during the oxidation of methane. The products of the reaction were hydrogen sulphide and, in the presence of iron, Fe S. Panganibar et al. (1979) found that bacteria found in the sediments of a fresh water lake could oxidize methane to carbon dioxide using sulphate ion as the terminal electron acceptor. Barnes and Goldberg (1976) reported the presence of methane oxidizing bacteria, possibly sulphate ion reducers, in anoxic marine sediments.

Several reactions have been proposed for anaerobic methane oxidation utilizing sulphate as electron acceptor.

$$Fe\,SO_4 + CH_4 \longrightarrow Fe\,S + CO_2 + 2H_2O$$ Zajic and Kosaric, 1974

$$Fe^{2+} + SO_4^{2-} + CH_4 \longrightarrow Fe\,S + CO_3^{2-} + 2H^+ + H_2O$$ Sorokin, 1957

$$SO_4^{2-} + CH_4 \longrightarrow S^{2-} + CO_3^{2-} + 2H^+ + H_2O$$ Sorokin, 1957

$$SO_4^{2-} + 2H^+ + CH_4 \longrightarrow H_2S + CO_2 + 2H_2O$$ Barnes and Goldberg, 1976

$$SO_4^{2-} + CH_4 \longrightarrow H_2S + HCO_3^- + H_2O$$ Zajic and Kosaric, 1974

The products of the reactions are sulphide salts, H_2S, or compounds that will react to produce these compounds.

The use of bacteria that use nitrate or sulphate ion as the terminal electron acceptor has the advantage that these ions, unlike oxygen, do not constitute an explosion hazard when mixed with methane, will not cause the spontaneous oxidation of coal, and can be injected into coal in an aqueous state. However, any process using sulphate

may form H_2S, which is corrosive and is toxic at low concentrations (Appendix F). Two grams of H_2S would be liberated for every gram of methane oxidized (Appendix F). In the presence of iron, 5.49 g of Fe S would be deposited in the coal and 9.49 g of Fe SO_4 would have to be present or injected into the coal (Appendix F). Any process requiring nitrate ion would require 4.21 g KNO_3 for every gram of methane oxidized (Appendix F). According to the equation suggested by Zajic and Kosaric (1974), two moles of hydrogen would be produced for every mole of methane oxidized in this reaction. The production of another explosive gas as a byproduct of the reaction would not reduce the threat of explosion.

Thus there are potentially two electron acceptors which can be utilized for anaerobic methane oxidation. Of the two, only sulphate ion has been found in coal (Khenkina et al. 1977). Coals, in general tend to have very low levels of nitrate ion (Kurdish et al. 1978, Khenkina et al. 1977).

2.2.10 Physical Characteristics

The size of MOM'S may limit the situations where the organisms can be used for biological methane control.

The size of the microorganism is of no consequence if the organism is applied directly to the surface of the coal. In cases where the microorganisms must pass through the coal structure, the size of the microorganism may limit its passage through the coal structure.

Organisms could pass into the structure of coal either by passing through native pores or by passing through fractures in the coal. Studies on the ability of microorganisms to pass through a porous medium have shown that the pores must be as large as the organism for passage to occur (Kalish et al. 1968). Bacteria have been shown to penetrate low permeability Berea sandstone cores at 230 psi (Kalish et al. 1964). In this study bacteria of differing sizes were injected into sandstone cores with a median pore radius of 3.5 to 4.0 μ and a minimum pore diameter of 0.5 μ. It was found that bacteria of formation pore size or less can penetrate formations easily with little or no loss of permeability in the core.

Coal structures can be permeated with pores and fractures. Pore sizes can range from less than 12Å up to 100 μ (Mikhail and Patching 1980, Cheng Tsai 1982). The majority of pores found in coal are probably 3.0 μ or less (Mahajan and Walker 1978), fractures ranging from 100Å to over 100 μ in diameter can also exist in coal (Mikhail and Patching 1980, Kissell and Bielicki 1973).

The pore and fracture systems of coal may limit the entry of certain microorganisms into coal. The majority of methane

oxidizing bacteria examined are similar in size to that of most coal pores (Table 17, Mahajan and Walker 1978). The methane oxidizing yeasts isolated by Wolf and Hanson (1979) are larger, ranging in size from 3 x 5μ to 3 x 7μ. The methane oxidizing fungus described by Zajic et al. (1969) is larger still. The hyphae of this organism were 1.5 to 4.5μ wide and up to several millimeters in length. Thus it would be reasonable to suggest that bacteria could penetrate most coals whereas yeast and fungi would only be able to penetrate highly fractured coals. This is supported by the evidence of Farrell and Turner (1932) who found that low permeability anthracite coals only contained bacteria when they were fractured. Fungi have only been found on the surface suggesting that even the fracture system was inadequate in allowing the organisms to penetrate the coal (Rogoff et al. 1962).

2.2.11 Heat Effects

The quantity of heat evolved by MOM'S during metabolic activity has not been established directly. A direct correlation has been shown to exist between the oxygen consumption of a microorganism and the heat evolved. Cooney et al. (1968) investigated the heat evolved during metabolism by yeast, a fungus, and 2 bacteria grown on different substrates and at different growth rates. They concluded that heat evolution was independent of growth rate, growth substrate, and type of organism. They showed that the heat produced by the organisms was proportional to the amount of oxygen consumed and that 3.44 Kcal were produced per gram of oxygen consumed. This relationship was found to be independant of the type of organism.

The heat evolved by MOM'S does not effect the growth of the organisms in systems where it is dissipated rapidly. If the heat is not rapidly dissipated, temperature increases will occur that will eventually kill the organisms. MOM'S will only grow within a range of about 25^{o} (Tables 10 and 12). In substances that have low net thermal conductivities, temperature increases of this order could occur due to the metabolic activity of the organisms.

Two general cases will be examined. In the case of microorganisms actively oxidizing methane withing the coal structure the heat produced will diffuse with difficulty through the coal. In this case the organisms will be able to oxidize approximately 4.8 g of methane per Kg of coal before the temperature increases by 25^{o} C. This represents approximately 40% of the methane present in typical Canadian coals (see Appendix G, for specific examples and details). At present it is not known whether the organisms would be killed under these conditions or only halted in their activity. If the latter is the case then they would probably regain their activity once the temperature had returned to ambient.

In the second case the MOM'S would be active on the surface of exposed coal. Calculations shown in Appendix H show that the heat generated under these conditions will be dissipated without any problems as long as there is forced convection over the surface.

3.0 METHODS OF APPLICATION AND THEIR POTENTIAL LIMITATIONS

There are three possible modes for applying MOM'S. These are:

(i) direct infusion into coal seam
(ii) spraying of the organisms onto the surface of the coal
(iii) by use of a biological gas scrubber

The limitations of these methods will be discussed in the following sections.

3.1 Infusion

Water infusion of a coal seam is a well established process (Cervik 1976). Seams can be infused from the face or from areas above the seam (surface or road beds).

Thus getting the organisms into the seam would appear to offer no problems. However, this method would appear to suffer from two serious drawbacks. Firstly, the availability of oxygen is a serious problem for aerobic organisms (Section 2.2.8). Supplying this in the gaseous form may give rise to an explosion hazard. The other alternative is to supply the oxygen in another form. Two sources have been noted (Section 2.2.9), nitrate and sulphate ions. Nitrate is low in coal and so would have to be added in the infusion water thereby increasing the cost. Sulphate may be present in the coal but would most probably result in the formation of deadly hydrogen sulphide gas.

The second limitation to infusion is the possible heating of the coal so that the organisms would be killed off. Thus the reaction would have to be controlled by the use of limiting amounts of oxygen. This would result in much slower removal of methane than the rates quoted in section 2.2.3. The use of anaerobic organisms would not result in the same build up of heat as that which would occur using aerobic organisms.

In certain cases the infusion process may limit the working of the mine but, in general, the operation of the mine can be worked around the various infusion sites.

3.2 Surface Application

This method would appear to be the simplest to use and would certainly be the method of choice for initial field tests. The only apparent limitation to this method would be in applying enough of the organisms to ensure adequate removal rates. Heating

effects and oxygen availability would not be a problem. Ionic constituents leaching from the coal may be troublesome but resistant strains could be selected.

3.3 Biological Gas Scrubber

A third method for applying the organisms would be to have them immobilized in a scrubbing tower placed in close proximity to the source of methane. Air from the mine face would be circulated through the tower where the methane would be oxidized. Such a system could be used in situations where ionic contaminants in the coal have the potential to poison the MOM'S.

4.0 APPLICATIONS IN COAL STORAGE AND TRANSPORTATION SYSTEMS.

In general, the use of the organisms in these systems would have the same limitations as those found in coal mines. The two prime limitations which would have to be overcome would be availability of oxygen and temperature.

As described previously, MOM's require large amounts of oxygen in order to oxidize methane. Thus completely closed systems will rapidly use up the available oxygen and further oxidation would cease. Thus there must be some method to allow controlled access of oxygen for complete removal of methane. Anaerobic organisms may be the answer, especially if nitrates could be added to the coal. The use of sulphate as an oxygen source would not be recommended.

The second limiting factor would be temperature effects, especially in the winter. There are two ways this can be overcome. Firstly, it should be possible to select for psychrophillic (cold loving) methane oxidizing bacteria. Such organisms should be active in the sediments of large, cold water lakes and in Arctic deltas. Such organisms should be quite active down to a few degrees below $0^{o}C$.

A second alternative would be to use a biological gas scrubber which is heated to between 25 and 45 ^{o}C where most mesophilic organisms are active.

5.0 Conclusions

A review of the scientific literature shows that microbiological control of methane in coal mines in feasible. This is supported by reports of both field tests and laboratory experiments.

A number of parameters were studied to see which, if any, would limit the effectiveness of the method. The following conclusions could be drawn:

A. Methane oxidizing bacteria have been field tested in at least two countries, Russia and India.

B. The physiological properties of methane oxidizing microorganisms were consistent with the conditions found in coal mines. In particular the following parameters were found to be favorable for their use.

(i) Temperature

The temperature optima for most methane oxidizers fall in the range 15-40°C which is within the range found in most coal mines. For cooler temperature applications (storage silos and shipholds) psychrophilic organisms could be isolated and used.

(ii) pH

The pH optima for these organisms were found to be between 5.0 and 8.0 which is similar to the pH of most mine waters studied.

(iii) Pressure

Pressure effects were not considered to be a factor since a large variety of organisms were shown to be capable of being subjected to high pressures without showing adverse effects.

(iv) Ion Effects

Because of the various ionic requirements of the methane oxidizers, organisms would be isolated from waters associated with the particular mine chosen for potential application so that they would be acclimatized to the particular ionic environment.

(v) Oxygen Requirements

The study concluded that aerobic organisms could not be used for infusion of a coal seam. However, if anaerobic organisms were available they could be used with a suitable electron acceptor e.g. nitrate or sulphate ions. Oxygen would not be a problem for surface application and for use in non-sealed storage containers.

(vi) Physical Characteristics

Bacteria would be the organisms of choice since their smaller size would permit better penetration of the coal structure.

C. Biological oxidation of methane could be applied in the following mining situations.

(i) Hydraulic mining. The organisms could be added to the cutting fluids.

(ii) Surface application. The organisms could be sprayed on gobs and other non-worked areas.

(iii) Storage and transportation systems. Organisms could be sprayed onto coal in ventilated storage and transportation systems. In cases where temperatures were low either pschrophilic organisms could be used or heated biological scrubbing towers could be designed.

The only situation in which it would not be practical to use these organisms would be in the infusion of cultures ahead of a working face. In this situation the availability of oxygen would be the limiting factor since the solubility of oxygen in water, even at high pressure, does not allow a high enough concentration to oxidize all the methane present. Anaerobic methane oxidation may be applicable if a suitable electron acceptor was readily available at low cost e.g. nitrate or sulphate ions.

6.0 RECOMMENDATIONS FOR FURTHER RESEARCH AND DEVELOPMENT

Based on the findings of this study it is recommended that the following research program be carried out:

Phase IIa - Isolation of methane oxidizing microorganisms

Phase IIb - Optimization of methane oxidizing activity

Phase III - Construction and running of a dynamic model for *in situ* microbial methane control.

6.1 Phase II a and b. Isolation and Optimization

Methane oxidizing micoorganisms should be isolated from Western Canadian coal mines. The organisms can either be isolated from the coal or from any associated mine water. The organisms should be cultured in media which closely resembles the coal mine water and/or the soluble components found in the coal.

The organisms which are isolated from the mines will have their culture conditions optimized for maximum methane oxidation. The organisms exhibiting the highest methane oxidizing activity will then be grown in large quantities for testing in the dynamic test rig.

6.2 Phase III. Dynamic Modelling

The best methane oxidizing organisms should be tested in a dynamic test rig. Such a rig should consist of an air tight chamber (tube shaped) fitted with both inlet and exit valves. Coal would be placed in this tube and air passed over it. Measurement of the methane content in the effluent gas will give a measurement of the rate of methane effusing from the coal. Varying amounts of microbial cultures should be added to the coal and the levels of methane in the effluent gas measured again. This will allow the measurement of the reduction in methane content of the air due to microbial oxidation.

The optimum number of bacteria, the effect of various additives and longevity of their metabolic activity will be determined.

It is anticipated that such a research project can be carried out in approximately twelve months.

On successful completion of the above study further work on in situ testing under actual mine conditions and design and testing of a bio-scrubber should be carried out.

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APPENDICES

Appendix A: Volumes of air required by methane oxidizing microorganisms to oxidize methane in coal.

Methane oxidizing microorganisms require from 0.29 to 1.8 moles of oxygen/ mole of methane oxidized (Table 16). Therefore from 7.13 to 44.28 g of oxygen must be injected into a coal containing 12.30 g of methane / kg of coal for methane oxidizing microorganisms to oxidize all of the methane in the coal. At 1 atmosphere pressure and standard temperature, 4.99 to 31.0 litre of oxygen or 23.76 to 147.6 litre of air /kg coal would have to be injected into the coal seam for the methane oxidizing microorganisms to be able to oxidize all of the methane in the coal.

Appendix B: Oxygen solubilities in water at varying pressures.

At 1 atmosphere of air pressure 9.0 mg of oxygen / litre of water at 20 C and 5.69 mg of oxygen / litre of water at 37 C is soluble in air saturated fresh water. (Hitchman, 1978). Assuming a range of oxygen consumption / mole of methane oxidized by methane oxidizing microorganisms of 0.29 to 1.8 mole oxygen consumed / mole methane oxidized (Table 1.) this liquid would contain enough oxygen to oxidize 2.5 to 15.4 mg of methane /litre of water at 20 C and 1.85 to 11.54 mg of methane / litre of water at 37 C.

At this pressure, if a coal was infused with 3 % w/w (See section 5) water and contained the highest levels of methane found in American coals, 12.30 g methane / kg coal (Hitchman 1978), these amounts of oxygen would allow methane oxidizing microorganisms in the coal to oxidize only 2.1×10^{-4} to 5.5×10^{-3} % and 4.5×10^{-4} to 2.8×10^{-3} % of methane in the coal at 20° C and 37° C resepctively.

At a pressure of 2000 p.s.i or 136 atmosphere at 37° C, 1.2 % oxygen will dissolve in a litre of water. (Battino and Clever, 1966). Assuming a range of oxygen consumption / mole of methane oxidized by methane oxidizing microorganisms of 0.29 to 1.8 mole oxygen consumed / mole methane oxidized (Table 1.), this liquid would contain enough oxygen to allow methane oxidizing microorganisms to oxidize 1.19 to 7.40 g methane / litre of water at 37° C.

At this pressure, if a coal was infused with 3 % w/w water (See section 5) and contained the highest levels of methane found in American coals, 12.30 g methane / kg coal (Hitchman, 1978) the amount of oxygen dissolved in the water would allow methane oxidizing microorganisms in the coal to oxidize from 0.29 to 1.8% of the methane in the coal at 37° C.

Appendix C: Air flows and the supply of oxygen to methane oxidizing microorganisms.

The amounts of oxygen (as air) required by a microorganism found on the face of coal that is oxidizing methane emitted by the coal will depend on the ratio of oxygen consumed by the microorganism / g methane oxidized, the maximum rate of oxidation of methane by the microorganism, and the emission rate of the methane.

The rate of methane emission by the coal face will be important with respect to maximum rates of oxygen consumption only if the rate of emission is less than the maximum rate of oxidation by the organism. The measurement of rates of gas emission from coal faces is difficult due to the large variations of emission rates found even in the same coal (Hargraves, 1973). We shall assume for this discussion that the rate of methane emission exceeds the maximum rate of methane oxidation by a microorganism.

The maximum rate of methane oxidation by a methane oxidizing microorganism will be assumed to be 5.83 g / g methane oxidized /h/ litre of culture (Section 4.3) and the ratio of oxygen consumed/ mole methane oxidized is from 0.29 to 1.8 mole oxygen consumed / mole methane oxidized (Table 16). If a square metre of a coal surface has 1 L of culture with the above characteristics applied to it (10 % w/w if the microorganisms penetrate / cm into the coal face) the microorganism will oxidize 5.83 g methane / h / m^2 of coal face and require 3.38 to 20.99 g of oxygen / h / m^2 of coal face or 11.27 to 69.97 litre air / h /m^2 of coal face.

If the microorganism has complete access to the oxygen in the air upto 10 cm from the surface of the coal then an air velocity of 0.11 to 0.70 m / h over the coal face would be necessary to supply enough oxygen to the microorganism to oxidize 5.83 g of methane, emitted from the coal face / h / m^2 of coal face.

Appendix D: The effects of gases on humans

Some gases present in mines, as well as the gases that are products of bacterial metabolism, can have effects on the health of humans. The effects of each gas are reviewed below :

Methane - Methane has no physiological effect on humans. (Gibbs and Pitus, 1978)

Oxygen - The normal level of oxygen in the air is 20 - 21 % volume in air. Under conditions that reduce the oxygen content of air such as the aerobic oxidation of methane physiological effects will occur. At oxygen level below 10 % by volume in air, breathing difficulties, nausea, and vomiting can occur. At oxygen levels below 6 % by volume in air breathing will cease. (Gibbs and Pintus, 1978)

Carbon dioxide - Carbon dioxide is the final metabolic product of methane metabolism and is emitted by some coal seams. At high concentrations, above 2 % by volume in air it can cause increased respiratory ventillation causing lung collapse and by displacing air cause asphyxiation (Gibbs and Pintus, 1978)

Hydrogen Sulphide - Hydrogen sulphide is the major final metabolic product of anaerobic methane oxidizing bacteria that use sulphate as a terminal electron receptor. At concentrations of 1000 ppm it can cause respiratory paralysis and death within one hour (Workers, Health, Safety, and Compensation, Occupational Health and Safety Division, Government of Alberta, 1983)

Appendix E: Oxygen availability in closed systems.

Coal can occupy from 0 to 100 % of the space in a coal transportation or storage system. A system of volume 1 m containing 1% v/v coal will contain, at standard temperature and pressure, 207.9 litre or 297 g of oxygen. This will be all the oxygen available to methane oxidizing microorganisms present in the system if the system is closed. If the methane oxidizing microorganisms present in the system require from 0.29 to 1.8 moles of oxygen/mole of methane oxidized (Table 15), then the oxygen supply in the system would allow the microorganisms to oxidize from 82.5 to 512.1 g of methane.

The density of coal can range from 1.1 to 1.6 g/cm in ash free coal (Van Krevelen, 1961). A system of volume 1 m containing 1% v/v coal containing 12.3 g methane / kg coal, and having a density of 2.25 g/cm would contain 271.8 g of methane. If the system ontained methane oxidizing microorganisms that consumed from 0.29 to 1.8 moles of oxygen/ mole of methane oxidized, from 30.4 to 100% of the methane present in the coal could be oxidized by the microorganisms using the oxygen present in the system. At coal contents of the system exceeding about 2% v/v the proportion of methane potentially oxidized by methane oxidizing micoorganisms falls rapidly. If the system contained 10% v/v coal, 270 g of oxygen would be available to the microorganisms and only from 75 to 465.5 g of the 2763 g of methane present in the system could be oxidized by the microorganisms present or sent in the system. If the system contained 50% v/v coal, 1485 g of oxygen would be available to the microorganisms and only from 41.2 to 250 of the 13,530 g of methane present in the system (up to 1.85%) could be oxidized by the microorganisms present in the systen.

Appendix F: Sulphate and nitrate ion requirements of anaerobic methane oxidizing microorganisms.

By using the following reaction outlined by Zajic and Zajic (1974), for the reduction of nitrate ion during the oxidation of methane by a methane oxidizing microorganism,

$$CH_4 + 2NO_3^- \longrightarrow CO_2 + N_2 + 6H_2O$$

we see that 2 moles of methane are oxidized by microorganisms for every 2 moles of NO_3 (as KNO_3) reduced. Therefore 4.21 g of KNO_3 /g CH_4 oxidized would need to be supplied to a microorganism oxidizing methane. In a coal seam containing 10 g CH_4 /kg coal, 42.1 kg of KNO_3 /tonne coal would need to be supplied to the microorganisms to enable them to oxidize all the methane present.

By using the following reaction outlined by Zajic and Zajic (1974), for the reduction of sulphate ion in the presence of iron during the oxidation of methane by a methane oxidizing microorganism

$$CH_4 + FeSO_4 \longrightarrow FeS + CO_2 + 2H_2O$$

we see that 1 mole of methane is oxidized and 1 mole of Fe S is produced for every mole of sulphate ion reduced. Therefore 5.49 g of Fe SO_4 /g CH_4 oxidized would need to be supplied to a microorganism oxidizing methane, and 5.49 g of Fe S/g CH_4 oxidized would be deposited in the coal. In a coal seam containing 10 g CH_4 /kg coal, 94.9 kg of Fe SO_4/ tonne of coal would need to be supplied to the microorganisms to enable them to oxidize all the methane present in the coal and 54.9 kg of Fe S/ tonne of coal would be deposited in the coal.

By using the following reaction outlined by Barnes and Goldberg (1974) for the reduction of free sulphate ion during the oxidation of methane by methane oxidizing microorganisms

$$SO_4^{2-} + 2H^+ + CH_4 \longrightarrow H_2S + CO_2 + 2H_2O$$

we see that 1 mole of H_2S is produced for every mole of methane oxidized. Therefore 2.13 g of H_2S /g CH_4 oxidized would be produced. In a coal seam containing 10 g CH_4 /kg coal, 21.3 kg of H_2S / tonne coal would be produced.

Appendix G: Net heating effects in a closed system due to heat evolution

When a methane-oxidizing microorganism is injected into coal, the methane will be oxidized and heat will be released. If conduction of heat through the coal and the diffusion of gases through the coal are neglected temperature increases in the coal will be directly proportional heat output.

If we make the following assumptions:

1. Heat evolved by organism - 3.44 Kcal / g oxygen consumed (Cooney **et al.** 1968).

2. Specific heat of coal 0.25 to 0.38 cal/g coal/°C. (Perry, 1941)

3. Oxygen consumption - 0.29 to 1.8 moles oxygen consumed / mole methane oxidized (Table 16)

4. Maximum temperature range of methane oxidation by oxidizing organisms. - 25° C (Tables 9 and 11)

5. Culture content of coal. 3 % (w/w)

The temperature increase in a solid is governed by the equation :

$$T = Q / C$$

T = temperature (°C)
Q = heat evolved in material (Kcal/kg)
C = specific heat of material (Kcal/kg °C)

At the specific heat of 0.25 Kcal/ kg °C a heat input of 6.25 Kcal / kg coal will raise the temperature of the coal 25° C. Methane oxidizing microorganisms consuming 0.29 to 1.8 moles oxygen / mole of methane oxidized and evolving 3.44 kcal / g oxygen consumed will be able to oxidize from 0.80 to 3.13 g of methane / kg of coal before the temperature of the coal will increase 25° C. If the coal contained 12.3 g methane /kg of coal, the methane oxidizing microorganisms

under these conditions would oxidize from 4.1 to 25.4% of the methane in the coal.

At the specific heat of 0.38 cal/ g coal / °C. a heat input of 9.5 Kcal / kg coal will raise the temperature of the coal 25° C. Methane oxidizing microorganisms consuming from 0.29 to 1.8 moles oxygen/ mole of methane oxidized and evolving 3.44 Kcal / g oxygen consumed will be able to oxidize from 0.77 to 4.76 g of methane / kg coal before increasing the temperature of the coal 25° C. If the coal contained 12.3 g methane / kg of coal, the methane oxidizing microorganisms under these conditions would oxidize from 6.3 to 38.7 % of the methane in the coal.

Appendix H: Convective heat cooling from a coal surface

Heat can be transferred from a surface by forced or natural convections. Both forced and natural convection heat transfer is determined by the Newton rate equation (Welty, 1974):

$$q = hA (T_s - T_f) \qquad (T_s - T_f) = \Delta T$$

q = rate of heat transfer in Kcal/hr

A = area of surface in m^2

h = convective heat transfer coefficient in Kcal/h . m^2 . °C

T_s = temperature of surface in °C

T_f = temperature of gas over surface

The value of h varies with the nature of the air flow over the surface, the nature of the surface, and the nature of the gas flowing over the surface. For the free convection of air, values of h from 4.4 to 24.4 (Kothandaraman and Subramanyan (1975) and 1.5 to 15 (Welty 1974) have been reported. For forced convection of air values of h from 9.75 to 2.44 (Kothandaraman and Subramanyan, 1975) and 7.5 to 150 (Welty 1974) have been reported. The minimum values for forced convection occur when air flow is barely detectable over a surface.

When a methane oxidizing microorganism is applied to the surface of a coal face it will begin to oxidize methane emitted from the surface and evolve heat. This will raise the temperature of the surface of the coal above that of the air flowing over it. Heat will then be transferred to the air, and the ΔT will stabilize at some point. Given air flow generating an h value of the minimum reported, 7.5, a minimum value of h of 1.5 for unventilated areas, and the rate of heat evolution by methane oxidizing microorganisms found on the surface, we can calculate the $T_{surface}$ - T air

needed to dissipate the heat being generated by the methane oxidizing microorganisms on the surface of the coal.

Under the conditions described in Appendix C methane oxidizing microorganisms could oxidize up to 5.83 gm methane/m^2 of coal/h. If the microorganisms utilize from 0.25 to 1.8 moles of oxygen/mole of methane oxidized (Table 10) and evolve 3.44 Kcal of heat/g oxygen utilized (Cooney,1963), they will evolve from 11.6 to 72.2 Kcal/m^2 of coal face/h. The temperature of the coal face must, for forced air conditions, be from 1.5 to 9.6° C higher than the air and under natural convection conditions must be from 7.7 to 40.1° C higher than the air for complete heat dissipation to occur.

Appendix I: Rates of water infusion into coal.

Infusion rates from 0.5 to 2.0 litre of water/minute can be acieved without fracturing coal (Deprez, 1978). If a coal with a density of 2.2 g/cm^3 is infused with 3 % w/w water, each m^3 of coal will be infused with 66 kg of water over a period of time from 33 to 122 minutes.

Ideally infusion fronts in coal from a point infusion source will expand as a sphere until the width of the infused volume is greater than the seam thickness (Cervik, 1975). At this point any differences in permeability between the coal and the strata surrounding the seam will alter the shape of the infused area.

In the simplist case, where the effects of the surrounding strata on the shape of the infused area are ignored, the shape of an infused area of coal will be a sphere. The volume of this coal will be $\frac{4}{3} \pi R^3$ where R is ½ the seam thickness. At an infusion rate of 33 to 122 minute/m^3 of coal, the time required to infuse a sphere of coal 5m in diameter to 3 % w/w water would take 1.50 to 5.55 days.

Appendix J: Additional Tables.

Table 3: Non-bacterial microorganisms capable of oxidizing methane

Organism	Reference
5 strains of yeasts of genera **Sporobolomyces** and **Rhodotorula**	Wolf and Hanson,1979 Wolf and Hanson,1979
Fungus of **Graphium** genus	Zajic **et al.**,1969
Green algae of **Chlorella** genus	Enebo,1967
5 strains of yeasts of genera **Candida** **Torulopsis**, and **Rhodotorula**	Smirnova and Golubeva,1967

Table 4 Environmental sources of methane oxidizing bacteria

Source	Country of Isolation	Number of Strains isolated[1]	References
Garden soil	England	-	DeBont and Mulder,1974
Lowland marsh	U.S.S.R	4	Nansaraev and Zavarzin,1972
Soils,silts from oil refineries,soils from oilfields and gas fields, lake and river silts marsh soils	U.S.S.R	66	Smirnova and Golubeva,1969
Mud and water samples from ponds rivers, streams and ditches	U.K.,U.S.S.R France,Germany North America Egypt, North Africa	>100	Whitterbury et al.,1970
Soil	Sweden	-	Hamer et al.,1967
Oilfield Soils, natural gas seep soils, pond mud, garden soil	Texas, U.S.A	-	Davis et al.,1964
Sewage	Texas, U.S.A	-	Foster and Davis,1966
Soil	Oklahoma, U.S.A	450	Zajic,1964
Fresh water lake mud from fresh lake	Germany	4	Naguib and Overbeck,1970
Mud, Fresh water	Wisconsin, U.S.A	2	Vary and Johnson,1967
Garden and Field soils, pond muds, sewage, aquatic plants	Texas, U.S.A	30	Leadbetter and Foster,1958
Garden soils, pond mud, creek mud, oil contaminated soils	Texas, U.S.A	-	Coty,1967
Storage container water	Montana, U.S.A	-	Johnson and Temple,1962

1: if mentioned and/or greater than one.

Table 4: Environmental sources of methane oxidizing bacteria (Contd.)

Source	Country of Isolation	Number of Strains isolated	References
Soil, ground water	U.S.S.R	10	Bogdanova,1965
Soil	Louisiana, U.S.A	-	Brown et al.,1964
Soil, ditch water Arden Soil	Nigeria, Netherlands	13	Hazeu,1975
Lake water,mud	Wisconsin, U.S.A	-	Patt et al.,1974
Soil, plant leaves	Illinois, U.S.A	-	Wolnak et al.,1967
[illegible]	New York, U.S.A	-	Wertlieb and Vishniac,1965
[illegible]	Wisconsin, U.S.A	-	Sheehan and Johnson,1971
Soil, water samples	Hungary	35	Halasz and Simek,1968
Marine mud, water mud, soils, beach sand	U.S.A	229	Hutton and Zobell,1949
Lawns	Vancouver, British Columbia	-	Mueller,1969a
Woods	Vancouver, British Columbia	-	Mueller,1969b
Soils	South East Alberta	-	Roth-wood,1983
Coal mine waters	U.S.S.R	57	Ivanov et al.,1978
Soils, coal mine waters	U.S.S.R	15	Malashenko et al.,1975
Soils and coal mines	U.S.S.R	-	Malashenko and Romanovskaya,1976
Coal mine waters	Poland	200	Goldlewska - Lipowa,1979

.: It continued and/or greater than one.

Table 5: [illegible] oxidizing microorganisms

Source	Country of Isolation	Organism	Number of strains Isolated	Reference
Lake water	Wisconsin, U.S.A. and Lake 227, Ontario	yeast	[illegible]	Wolf and Hanson,1979
Sewage	London, Ontario	fungi	-	Zajic et al.,[illegible]
[illegible]	Sweden	algae	-	Ericson,1967
Soil	U.S.S.R.	yeast	3 genera	Smirnova and Golubeva,1969

1: If mentioned and/or if greater than one.

Table 6: Distribution of methane oxidizing bacteria[1]

Samples	# Samples examined	# Samples containing methane oxidizing bacteria	% Samples containing methane oxidizing bacteria
Lake, reservoir and river silts	10	6	60
Marsh soils	7	6	85.7
Soils from gardens, woods, plowland, and indoor plants	17	14	82.9
Soils contaminated with oil and oil products	16	12	75
Soils from gas fields	22	17	77.2
Soils from oil fields	37	23	62.2

1: From Smirnova and Golubeva, (1969)

Table 7: Distribution of methane oxidizing bacteria

Samples	# Samples examined	# Samples containing methane oxidizing bacteria	% Samples containing methane oxidizing bacteria
Marine mud	38	22	57.9
Brackish water mud	53	37	69.8
Serpentine earth	9	9	100
Oil-or gas-field soil	187	139	74.3
Lake Sanit	12	2	16.7

From Kuznetsova and ZoBell (1949)

Table 8: Microbial forms found associated with Coal or related to Coal Studies[1]

Organism		Normal habitat
A - Fungi		
Aspergillus minor		soil, dry vegetable matter
Penicillium	sp.	soil
Verticillium	sp.	soil
Trichoderma	sp.	soil
Fusorium	sp.	soil
Mucor	sp.	soil, manure, fruits
B - Bacteria		
Clostridium perfringens		soil, sewage, feces
Bacillus megaterium		soil, water, decomposing materials
Bacillus subtilus		soil, decomposing materials
Pseudomonas aeruginosa		soil, water, and sewage
Sarcina	sp.	soil, mud, swamp water, air
Gallionella	sp.	iron - containing water
Thiothrix	sp.	water environments where H_2S occurs

1: From Rogoff et al. (1962)

Table 9: 3 C or greater compounds utilized by some non-obligate methanotrophic bacteria as sole carbon sources

Organism	C Compounds	Reference
[illegible]	Propane, butane, n - pentane, n-heptane, ethanol, proponal, hexanol, heptanol, octanol, nonanol, decanol, propionic acid, caprylic acid, capric acid, glucose	Bewersdoff and Oostalek,1971
[illegible]	glucose, ethanol, propane, n - butanol.	Vary and Johnson,1967
2 unidentified strains	paraffin oils	Naguib and Overbeck,1970
3 unidentified strains	glucose, galactose, sucrose, lactose	Namsaraev and Zavarzin,1972
2 unidentified strains	propane, butane, kerosine, solid paraffins, glucose, glycerol, galactose, sucrose, starch, phenols napthalene	Bogdanova,1965

Table 10: Temperature range of growth for various methane oxidizing bacteria

Organism	Temperature range of growth or temperature grown at (°C)	Reference
Methylomonas methanica (type strain)	20 - 35	Leadbetter,1974
Methylomonas methanoxidans (type strain)	20 - 40	Leadbetter,1974
Methylomonas methanitrificans (type strain)	20 - 35	Leadbetter,1974
Methylococcus capsulatus	30 - 50	Leadbetter,1974
[illegible]	25 - 30	Vary and Johnson,1967
[illegible] (mixed culture)	45	Sheehan and Johnson,1971
[illegible] isolates (two groups)	30 - 45 37 - 42	Malashenko **et al.**,1975
1	33	Halasz and simek,1968
[illegible] strains	15 - 35	Hutton and ZoBell,1949
Bacillus sp.	20 - 37	Wolnak **et al.**,1967
[illegible]	25 - 40	Bewersdorff and Dostalek,1971
Methylomonas methanoxidans strain Brown and Strawinski	20 - 45	Brown **et al.**,1964
13 strains	20 - 40	Hazeu,1975
Methanomonas sp.	25	Hamer **et al.**,1967

Table 10: Temperature range of growth for various methane oxidizing bacteria (Contd.)

Organism	Temperature range of growth or temperature grown at (°C)	Reference
Methanomonas capsulatus	37 - 47	Harwood and Pirt,1972
10 strains of genus Mycobacterium, Pseudobacterium Pseudomonas, and Proactinomyces	28 - 33	Bogdanova,1965
Methylococcus capsulatus	30 - 50	Foster and Davis,1966
41	28	DeBont and Mulder,1974

Table 11: pH range of growth for various methane oxidizing bacteria

Organism	pH range of growth or pH grown at	Reference
Methylomonas methanica (type strain)	6.0- 8.0	Leadbetter,1974
Methylomonas methanoxidans (type strain)	5.0- 7.0	Leadbetter,1974
[illegible]	6.0- 7.46	Vary and Johnson,1967
[illegible] (mixed culture)	6.0- 7.0	Sheehan and Johnson,1971
[illegible]	6.[illegible]	Halasz and Simek,1968
[illegible] strains	4.5- [illegible]	Hutton and ZoBell,1949
Several isolates (= undefined)	5.4- 9.5	ZoBell,1946
Bacillus sp.	6.4- 7.[illegible]	Wolnak **et al.**,1967
[illegible]	4.9- 7.0	Bewersdorff and Dostalek,1971
Methylomonas methanoxidans strain Brown and Strawinski	5.0- 5.0	Brown **et al.**,1964
15 strains	6.[illegible]	Hazeu,1975
Methanomonas sp.	6.0	Hamer **et al.**,1967

Table 11: pH range of growth for various methane oxidizing bacteria (Contd.)

Organism	pH range of growth or grown at	Reference
Methanomonas capsulatus	4.2- 7.0	Harwood and Pirt,1972
Methanomonas methanica	6.0- 9.5	Johnson and Temple,1962
4 strains of genus Mycobacterium Pseudobacterium, Psuedomonas and Proactinomyces	3.0- 10.0	Bogdanova,1965
>100 strains	5.6- 7.4	Whittenbury et al.,1970
"	6.8	DeBont and Mulder,1974

Table 12: pH and temperature ranges of growth for various non-bacterial methane oxidizing microorganisms

Organism	pH range of growth or pH grown at	Temperature range of growth or temperature grown at (°C)	Reference
5 strains of yeasts genera **Sporobolomyces** and **Rhodotorula**	3.5- 4.0	25- 42	Wolf and Hanson,1979 Wolf and Hanson,1980
Graphium sp. (fungi)	2.7- 7.0	28	Zajic **et al.**,1969
Green algae of **Chlorella** sp.	7.0- 8.3	-	Enebo,1967
3 strains of of yeasts of genera **Candida**, **Torulopsis** and **Rhodotorula**	4.5- 6.5	29- 30	Smirnova and Golubeva,1969
	4.5- 8.3		Hutton and ZoBell,1949

Table 13: Multiplication of terrestrial microorganism after 48 hrs incubation at 30° C at different hydrostatic pressure.[1,2]

Organism	Hydrostatic pressure in atmospheres				
	1	300	400	500	600
Alkaligenes viscosus	4+	3+	2+	-d	-d
Bacillus alvei	4+	3+	+	-	-
Bacillus brevis	4+	3+	+	-	-d
Bacillus cereus	4+	4+	-	-	-
Bacillus circulans	4+	4+	-	-	-
Bacillus megatherium	4+	4+	+	-	-d
Bacillus mesentericus	4+	4+	3+	2+	-d
Bacillus mycoides	4+	4+	+	-	-d
Bacillus subtilus	4+	2+	+	-	-
Clostridium chauvei	4+	4+	2+	-	-
Clostridium histolyticum	4+	4+	3+	-	-d
Clostridium putrificum	4+	4+	2+	-	-
Clostridium septicum	4+	2+	+	-	-
Clostridium sporogenes	4+	3+	2+	-	-
Clostridium welchii	4+	4+	+	-	-
Escherichia coli	4+	4+	3+	2+	-
Micrococcus lysodeikticus	4+	2+	-	-d	-d
Mycobacterium phlei	4+	3+	2+	-d	-d
Mycobacterium smegmatis	4+	2+	+	-	-d
Proteus vulgaris	4+	2+	-	-	-d
Pseudomonas fluorescens	4+	4+	3+	-	-
Sarcina lutea	4+	3+	+	-	-d
Serratia marcescens	4+	-	-	-	-
Staphylococcus albus	4+	3+	3+	-	-
Staphylococcus aureus	4+	2+	-	-d	-d
Streptococcus lactis	4+	4+	4+	2+	-
Hansenula anomala	4+	2+	-d	-d	-d
Saccharomyces cerevisiae	4+	4+	-d	-d	-d
Saccharomyces ellipsoideus	4+	+	-d	-d	-d
Schizosaccharomyces octosporus	4+	-	-d	-d	-d
Sporobolomyces salmonicoler	4+	+	-d	-d	-d
Torula cremoris	4+	4+	2+	-d	-d

1: from Zobell and Johnson (1949)
2: plus signs show the degree of turbidity relative to the control culture at 1 atmo
minus signs indicate no growth.
d indicates that the culture had lost its ability to grow after decompression.

Table 14: **Ionic species present in media used to grow methane - oxidizing microorganisms**
Cation species present in the media

Organism	Reference	NO_3^-	Mg^{2+}	K^+	Fe^{2+}	Fe^{3+}	Ca^{2+}	Cu^{2+}	Zn^{2+}	Mn^{2+}	Co^{2+}	Mo^{2+}	Cl^-
Bacillus sp	Kulrak **et al.**,1967	+	+	+	+		+		+		+		+
T 10	Loveridge and Dostalek,1971	+	+	+		+	+	+	+		+		+
Methylococcus capsulatus	Foster and Davis,1966	+	+	+	+		+	+	+				+
HR	Vary and Johnson,1967	+	+	+		+	+	+	+		+		+
M45	Sneehan and Johnson,1967	+	+	+	+		+	+	+		+		+
Mixed culture	Mueller,1969a	+	+	+	+		+	+	+		+		+
Unnamed	Davis **et al.**,1964	+	+		+								
Pseudomonas methanica	Ferenci **et al.**,1975 Ferenci,1974 Higgins and Quayle,1970	+	+	+	+		+	+	+				+
Unnamed	Nagai **et al.**,1973		+	+		+	+	+	+				+
41	DeBont and Mulder,1974	+	+	+	+		+	+	+				+
Unnamed	Hammer **et al.**,1967	+	+	+		+			+			+	+
Methylococcus capsulatus	Harwood and Pirt,1972 (see above)	+	+	+		+	+	+	+		+		+
10 strains	Bogdanova,1965	+											

Table 14: Ionic specificity [illegible]

Organism	Reference	Mg^{2+}	Mn^{2+}	Fe^{2+}	Fe^{3+}	Ca^{2+}	Cu^{2+}	Zn^{2+}	Ni^{2+}	Co^{2+}	Co^{3+}	Pb^{2+}
Methylomonas methanoxidans	Brown **et al.**,1967	+	+	+	+	+	+	+	+	+	+	+
15 strains	Hazen,1975	+	+	+	+	+	+	+	+	+	+	+
50 strains	Leadbetter and Foster,1960	+	+	+	+	+	+	+	+	+	+	+
Unnamed	Patt **et al**,1974	+	+	+	+	+	+	+	+	+	+	+
5 strains of yeast	Wolf and Hanson,1979	+	+	+	+	+	+	+	+	+	+	+
Sporobolomyces and **Rhodotorula**	Wolf and Hanson,1980	+	+	+	+	+	+	+	+	+	+	+
Graphium sp (Fungi)	Zajic **et al.**,1969	+	+	+	+	+	+	+	+	+	+	+
3 genera yeasts (**Cardida**, **Torulopsis** **Rhodotorula**	Smirnova and Golubeva,1969	+	+	+	+	+	+	+	+	+	+	+
Green algae (**Chlorella** sp)	Erebo,1957	+	+	+	+	+	+	+	+	+	+	+

Table 14: (continued)

Organism	Reference	Anionic species present in the media							Nitrogen containing ions present in the media		
		SO_4^{2-}	PO_4^{3-}	SiO_3^{3-}	BO_4^{3-}	MoO_4^{2-}	SeO_3	Cl^-	NO_3^-	NO_2^-	NH_4^+
Bacillus sp	Wolrak **et al.**,19[illegible]	+	+	+					+		
T.10	Bewersdorft and Dostalek,1971	+	+					+			+
Methylococcus capsulatus	Foster and Davis,1966	+	+		+		+	+	+		
HR	Vary and Johnson,1967	+	+					+			+
H45	Sheehan and Johnson,1967	+	+			+		+	+		
Mixed culture	Mueller,1969a	+	+	+							+
Unnamed	Davis **et al.**,1964	+	+			+					
Pseudomonas methanica	Ferenci **et al.**,1975 Ferenci,1974 Higgins and Quayle,1970	+	+	+			+	+	+		
Unnamed	Nagai **et al.**,1973	+	+					+	+		
41	DeBont and Mulder,1974	+	+	+		+		+	+		
Unnamed	Hanner **et al.**,1967	+	+					+			+
Methylococcus capsulatus	Harwood and Pirt,1972 (see above)	+	+					+			+
10 strains	Bogdanova,1965	+	+					+	+	+	

Table 14:(continued)

Organism	Reference	Anionic species present in the media						Nitrogen containing ions present in the media			
		SO_4^{2-}	PO_4^{3-}	BO_3^{3-}	BO_4^{-}	MoO_4^{2-}	MoO_3	Cl^-	NO_3^-	NO_2^-	NH_4^+
Methylomonas methanoxidans	Brown **et al.**,1967	+	+					+	+		
16 strains	Hazeu,1972	+	+	+				+	+		
30 strains	Leadbetter and Foster,1958	+	+	+				+	+		
Unnamed	Patt **et al**,1974	+	+		+	+		+	+		+
5 strains of yeast	Wolf and Hanson,1979	+	+			+		+	0		+
Sporobolomyces and **Rhodotorula**	Wolf and Hanson,1980										
Graphium sp (Fungi)	Zajic **et al.**,1969	+						+			+
3 genera yeasts (**Cardida, Torulopsis Rhodotorula**)	Smirnova and Golubeva,1969	+	+					+	+		+
Green algae (**Chlorella** sp)	Enebo,1967	+	+					+	+		

1: ions also in this media but not in table CO_3^{2-}, $S_2O_3^{2-}$, S^{2-}.

Table 15: Commonly occuring minerals in coal [1]

Mineral	Approximate Formula	Mode of occurrence
Kaolin	$Al_2O_3.3SiO_2.xH_2O$	In Clay or shale
Muscovite	$K_2O.3Al_2O_3.6SiO_2.2H_2O$	
Biotite	$K_2O.MgO.Al_2O_3.3SiO_2.2H_2O$	
Calcite	$4CaO.3Al_2O_3.6SiO_2.H_2O$	
Quartz	SiO_2	
Montmorillonite	$(Fe,Mg,Ca)O.2(Al_2O_3).4SiO_2.2H_2O$	
Pyrite	$FeO.Al_2O_3.3SiO_2.2H_2O$	
Siderite	$3FeO.Al_2O_3.6SiO_2$	
Feldspar	$K_2O.Al_2O_3.6SiO_2$	
Anorthite	$CaO.Al_2O_3.2SiO_2$	
Hematite	Fe_2O_3	
Gypsum	$CaSO_4.2H_2O$	
Kaolinite	$Al_2O_3.2SiO_2.2H_2O$	
Chamosite	$(Fe,Mg,Fe)_5Al_2Si_3O_{10}(O,OH)_8$	

Pyrite	FeS_2
Marcasite	FeS_2
Calcite	$CaCO_3$
Aragonite	$2CaCO_3$
Grass-Stone	$2CaCO_3 . MgCO_3 . FeCO_3$
Siderite	$FeCO_3$
Witherite	$BaCO_3$
Gypsum	$CaSO_4$
Anhydrite	$CaSO_4$
Salt	NaCl
Apatite	$3Ca_3P_2O_8.CaF_2$
Spinel	$FeS_2 . FeS_2$
Zinc-blende	ZnS
Copper pyrites	$CuFeS_2$
Galena	PbS

As inclusions

nodules or

concentrations

Hematite Fe_2O_3

From Francis (1961).

Table 16: **Moles of oxygen utilized per mole of methane oxidized by various methane oxidizing microorganisms**

O_2 / CH_4 ratio [1]	Special Conditions	References
1.5	-	Cooney and Levine,1972
1.0 - 1.1	2 strains of bacteria	Whittenbury et al.,1970
0.4	30 strains of bacteria	Leadbetter and Foster,1958
0.6	-	Foster and Davis, 1966
1.6 - 1.7	methane or oxygen limited growth.	Nagai et al.,1973
1.6	2 strains, methane or oxygen limited growth	Vary and Johnson, 1967
1.1 - 1.2(a)	(a) actively growing cells	Naguib, 1976
1.4 - 1.8(b)	(b) same strain, resting cells	
0.29 - 0.31(a)	(a) Oxygen limited growth	Harwood and Pirt, 1972
1.8 (b)	(b) same strain, methane limited growth	
1.7	-	Bewers dorff and Dortalek, 1971
1.5	-	Sheehan and Johnson, 1971
1.1(a)	(a) 4 strains, all rapidly growing	Brown et el.,1964
1.7(b)	(b) same strains, resting cells	
0.6 [2]	4 yeast strains	Wolf and Hanson, 1979

1: ratio of moles oxygen consumed / moles methane oxidized
2: only reported case that is not of bacteria in this table

Table 17: Size ranges of various methane oxidizing bacteria

Organism	Size Range	Reference
[illegible]	rod, length 1 μ	Bewerdorff and Dostalek, 1971
Bacillus sp.	2-3u x 5-15 μ	Wolnak **et al.**, 1967
Methanomonas methanoxidans strain, Brown and Strawinski	1u x 1.5 - 3 μ	Brown **et al.**, 1964
Methylococcus capsulatus	coccus, 1 μ	Foster and Davis, 1966
Strain 41	rods, length upto 5u	DeBont and Mulder, 1974
[illegible]	0.3 to 1.5 μ x 1.3 to 3.0 μ	Halasz and Sinek, 1958
Unidentified strain	0.5u x 10 μ	Vary and Johnson, 1967
Unidentified strain	0.6 x 2 to 3 μ	Vary and Johnson, 1967
Unidentified strain	rod, length 2 - 4 μ	Davis **et al.**, 1964
Unidentified strain	0.5 μ x 2.0 μ	Nagai **et al.**, 1973
Methylomonas methanica type strain	0.5 μ x 1.0 μ	Leadbetter, 1974
Methylomonas methanoxidans type strain	1.0 μ x 1.5 to 3.0 μ	Leadbetter, 1974
Methylomonas methanitrificans type strain	1 to 2 μ x 2 to 4 μ	Leadbetter, 1974
Methylomonas capsulatus type strain	coccus, 1 μ	Leadbetter, 1974